DNAble® Molecular Detection Kit for *Cmm* in Tomato Tissue

Catalog No. DF 091 PT *Part # 12009*

Highlights:

- *Molecular detection of Cmm in tomato tissue*
- *Rapid amplification and detection in 10 minute assay*

Contents of DNAble Kit:

A. *RB1 Reaction Buffer*
B. *Cmm Master Mix*
C. *Flat Caps*
D. *MB1 Extraction Buffer*
E. *1.5 mL Extraction Tubes*
F. *Blue pestles*

Materials Not Provided:

- *3 mm Harris punch* (Part# 11209)*
- *Minipets: 5 µL and 50 µL* (Part# 11818, 11203)*
- *Marker*
- *8-well AmpliFire Reader* (Part# 11974)*

**available through EnviroLogix*

Intended Use

This test kit is intended for qualitative detection of *Clavibacter michiganensis michiganensis* (*Cmm*). The results of this test may facilitate rapid, point of need detection of *Cmm* in tomato plants.

How the Test Works

DNAble is an isothermal nucleic acid amplification technology enabling rapid amplification of a specific DNA target. In this test, samples are collected, processed, and added to the reaction buffer. The reaction buffer containing sample is then transferred to the lyophilized master mix, containing all the reagents needed to specifically recognize, amplify and detect the *Cmm*-specific DNA.

The amplified *Cmm*-specific DNA is detected in real-time and the results are displayed and interpreted within 10 minutes using our 8-well DNAble Reader.

Precautions and Notes

DNAble is a highly sensitive assay. Therefore the following precautions are recommended to reduce the chance of sample contamination:

- Clean the work stations and pipettes before and after use with 10% bleach solution
- It is recommended to physically separate sample preparation activities from DNAble assay activity
- Do not reuse kit disposables
- Use fresh pipette tips for each sample
- Discard used tips in a sealed container containing 10% bleach solution
- Use careful pipetting techniques to avoid cross-contamination between samples; avoid reaching over or pipetting over open tubes
- Wear disposable gloves and change between handling of samples

Important: Never open reaction tubes after reaction has occurred, as this will release amplified material into the environment and may contaminate subsequent reactions. Care should be taken when disposing of run reaction tubes to avoid possibility of tube leakage. Place completed reaction tubes back in original zippered pouch prior to disposal.

Kit Components

A. RB1 Reaction Buffer: Provided in green 8-well strip tubes (3 strips)

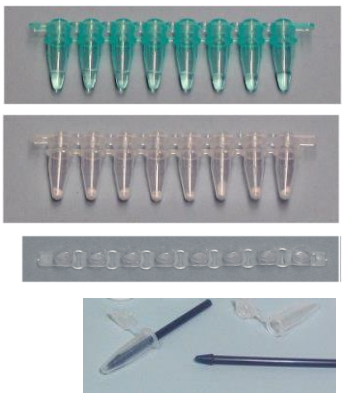
B. *Cmm* Master Mix: Lyophilized reagents provided in clear 8-well strip tubes (3 strips)

C. Flat Caps: used for capping the clear tubes prior to assay start (3 strips)

D. MB1 Extraction Buffer: One dropper bottle containing 15 mL of extraction buffer (for sample preparation)

E. 1.5 mL Extraction Tubes (25): One bag of 25 tubes for sample extraction

F. Blue Pestles (25): One bag of 25 pestles for sample extraction



Before Testing

- Remove needed DNAble Kit reagents from refrigerated storage. Allow reagents to come to room temperature before opening sealed white pouches.
- Ensure that all assay reagents, extracted sample, pipettes and flat caps are ready for use.
- Wear gloves and wipe gloves with disinfecting wipes between tomato tissue sample collection.

Tomato Tissue Sample Collection and Preparation

1. Sandwich a section of stem or leaf tissue between the cap of a closed extraction tube and the Harris Uni-Core punch and apply pressure to the stem while rotating the Uni-Core back and forth.

   Alternatively, harvest a section of stem, slice open lengthwise, and remove sample from tissue inside stem using Uni-Core punch as above.

   Note: Like all pathogen testing, sampling symptomatic tissue increases the likelihood of detecting Cmm *with DNAble.*

2. Release the cored sample in the 1.5 mL extraction tube by depressing the spring action plunger. Verify that the sample is at the bottom.

   Note: Clean the cutting edge of the Uni-Core punch between each sample: dip into 10% bleach solution (or other suitable sanitization solution), depress the plunger several times, then rinse with potable water.

3. Insert the pestle into the tube and grind the tissue by rotating the pestle against the sides of the tube with twisting motions. Continue this process for 5 seconds or until the plant tissue is gently ground to release bacteria.

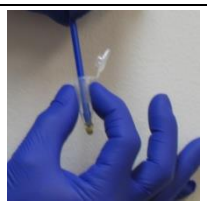
4. Holding the dropper bottle vertically, add 0.5 mL (to the 0.5 line on tube) of MB1 Extraction Buffer into the tube containing the tissue sample. Do not touch the dropper or pipette tip to the tube or tissue.

5. Repeat grinding step briefly to mix tissue with extraction buffer. Dispose of pestle.

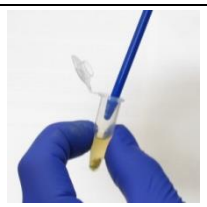
6. Using **red MiniPet** (or pipette set to 5 µL), **add 5 µL of sample extract to 1 well of the green reaction buffer strip** tube. Repeat with remaining samples and wells, changing pipette tips between samples.

Take tissue

Release tissue
in tube



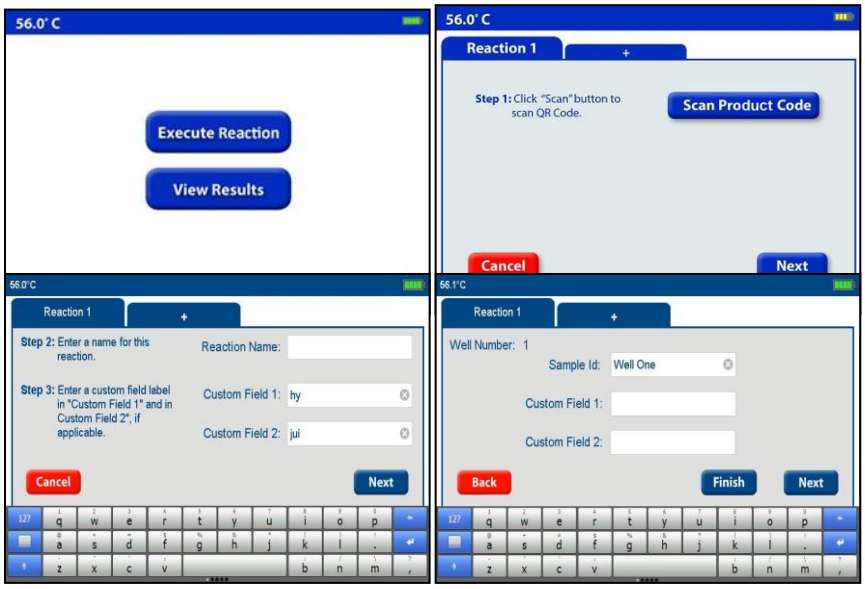
Grind tissue gently



Add buffer,
grind again briefly to mix.

Pipette 5 µL sample in one well
Change pipette tips between samples/wells

How to Run the DNAble Assay



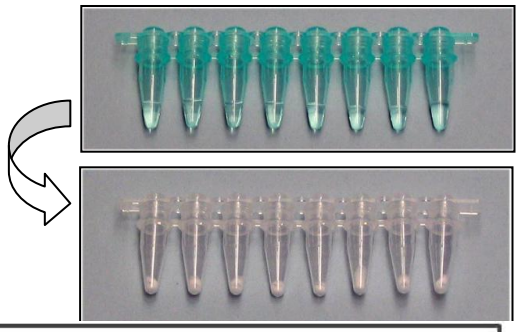
1. On the AmpliFire screen, select "**Execute Reaction**" then select "**Scan Product Code**". Use the barcode on the master mix foil pouch to scan the Cmm protocol on the 8-well AmpliFire Reader. Cmm_Lot # will display. Select "**Next**".

2. Under "Reaction Name" enter an appropriate reaction description**. This description is placed at the beginning of the file name.** Select "Next".

3. To enter sample specific information, add sample descriptions to the screens for Wells 1 through 8, clicking "Next" to advance to each Well. Select "Finish" to skip well-specific sample entry.

4. Remove clear Master Mix tubes from the foil pouch and gently tap down to ensure that the white pellet is at the bottom of the tubes.

   Important: *Mark flat cap for orientation of the clear Master Mix tubes (writing on clear tubes will interfere with results interpretation or leave marker residue in instrument).*



5. Using the **yellow MiniPet** or a multichannel pipette set to **50 µL**, transfer **50 µL from green strip tubes (containing sample)** to clear Master Mix tubes. Do **not** mix within the clear tube.

6. **Cap** Master Mix tubes with provided **Flat Caps** strip.

   Important: *Ensure that the tubes are* ***completely sealed*** *with flat caps*

7. Gently flick down on the resuspended, capped master mix to ensure that no bubbles are at the bottom of the tube and that master mix is fully resuspended.

8. Inspect tube to ensure that **no air bubbles are present within the sample volume** (a bubble at the top is fine) and that **cap is completely sealed.**

9. When the strip is ready select "**Start**". Place resuspended, capped clear strip tube in instrument and press "**Ok**".

10. After 10 minutes, the AmpliFire will produce a short beeping sound and display final results. Results will be interpreted as Not Detected (-) or Positive (+).

    Important: Positive results may be interpreted prior to assay completion, but the full assay time must be complete for complete result interpretation. (Empty wells will be interpreted as negative.)

11. After the assay is complete, carefully **remove run reaction strip tubes from instrument and place in opened foil pouch** (used to store master mix), seal and discard in waste container.

12. To export results, return to the home screen, then "View Results". Insert a USB storage device into instrument (left side) and select each run to export and "Export Selected" and "OK."The results will be saved in a PDF summary report as well as .csv file format.

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